Characterization of the Manganese Core of Reconstituted Ferritin by X-ray Absorption Spectroscopy

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The nature of the Mn/O/OH phase stabilized within reconstituted ferritin has been probed by X-ray absorption spectroscopy (XAS). Ferritin stabilizes Mn(III) as a metastable oxyhydroxide phase, and XAS establishes that the local environment of the Mn closely resembles that in γ- and β-MnOOH.

The synthesis of discrete nanometer-size inorganic particles with unusual structural and optical properties is currently of great interest in the developing field of nanotechnology. Several viable routes to these materials are available, often involving the use of constrained environments for inorganic precipitation. Thus, porous glasses,[1] zeolites,[2] reverse micelles,[3] and phospholipid vesicles[4] have been utilized as reaction cages for inorganic cluster formation. Recently, Meldrum *et al.* described a novel synthetic route based on the use of the supramolecular structure of the iron storage protein, ferritin.[5] This protein consists of a spherical polypeptide shell (apoferritin) surrounding a 6-nm inorganic core of the iron oxide, ferrihydrite, ($5Fe_2O_3{\cdot}9H_2O$).[6] Removal of the mineral core can be readily achieved by reductive dissolution and the resulting apoferritin subsequently reconstituted *in vitro* under controlled reaction conditions. Although previous work had implied that only iron oxides (ferrihydrite) could be synthesized within the protein cavity, Meldrum *et al.* were able to show that, under certain circumstances, the nucleation and growth of manganese oxyhydroxide could be specifically confined to the supramolecular cage.[5] Electron diffraction and lattice imaging studies have failed to detect any long range order, suggesting that the manganese oxyhydroxide core within ferritin is amorphous, although the product in the absence of the protein is a mixture of the crystalline oxides groutite (α-MnOOH) and hausmannite (Mn_3O_4).

Previously, XAS has been applied to determine the local structure about iron within the cores of ferritin and haemosiderins.[7] Therefore, we have employed XAS to investigate the local environment and the oxidation state of Mn in Mn-reconstituted ferritin.

Apoferritin was prepared from native horse spleen ferritin.[5,8] Samples of hausmannite (Mn_3O_4),[9] feitknechtite (β-MnOOH),[10] and manganite (γ-MnOOH)[11] were obtained, and their XAS were recorded to provide spectroscopic comparisons with data recorded for the core of Mn-reconstituted ferritin. Mn K-edge XAS were recorded at the Daresbury Synchrotron Radiation source.[12] The Mn K-edge shift from that of Mn metal was found to be +8.7 eV for Mn_3O_4; for β- and γ-MnOOH and the Mn-

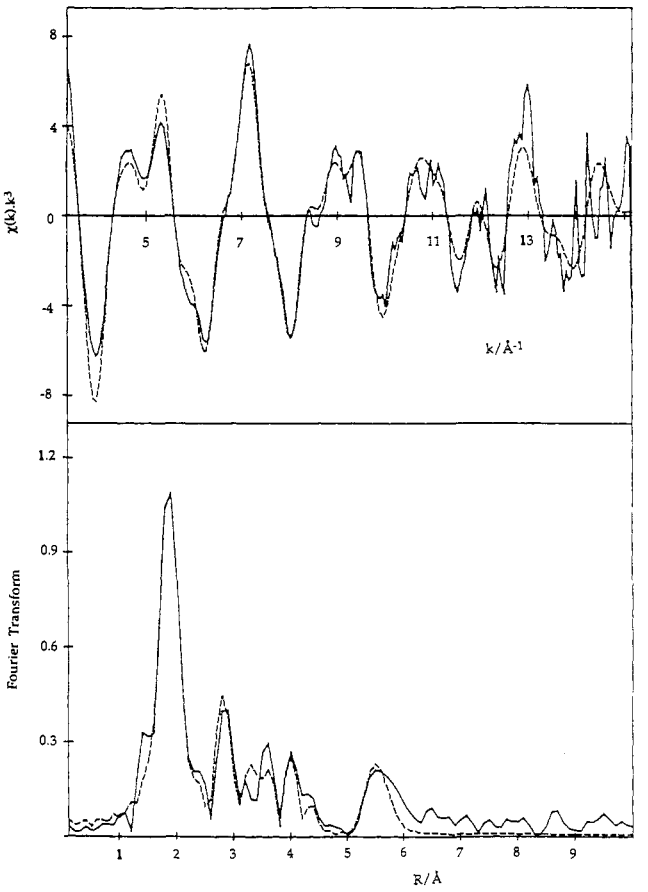

Figure 1. Manganese K-edge EXAFS of Mn-reconstituted ferritin (—) and a simulation (- - -) using the parameters given in Table I, together with their Fourier transforms.

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(8) Reconstitution with Mn(II) was achieved by addition of an aliquot of $MnCl_2$ solution to a buffered apoferritin solution (2.25×10^{-6} M apoferritin, 0.05 M pH 8.9 AMPSO = 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid)) such that the concentration of Mn(II) was 6.75×10^{-3} M. Oxidation was observed to occur over 24 h, as indicated by a change from colorless to brown. The buffer solution was then exchanged by dialysis against 0.15 M NaCl, and the resulting solution was concentrated to 50 mg of protein mL^{-1} using an ultrafiltration cell. Examination of the reconstituted protein solution by electron microscopy demonstrated that negligible nonspecific oxidation product was present.

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(12) Manganese K-edge X-ray absorption spectra were recorded at 60% harmonic rejection in transmission mode using three ion chambers at station 7.1 of the Daresbury Synchrotron Source, operating at 2 GeV with an average current of 150 mA using a Si(111) double crystal monochromator. The sample was positioned between the first two ion chambers, and a manganese metal foil was placed between the second and third ion chambers. Six scans were collected for the ferritin samples, two scans were recorded for both Mn_3O_4 and β-MnOOH, and one scan was recorded for γ-MnOOH. The data from the multiple scans were averaged. The samples were maintained at *ca.* 80 K during investigation. Background subtraction and data analysis were accomplished using the facilities of EXCURV 90 and related programs.[13] The manganese K-edge shift of the samples was measured relative to the manganese metal foil with the edge position being selected as the maximum of the first derivative. EXAFS data were analyzed using the single-scattering spherical wave approximation, and phase shifts were derived from *ab initio* calculations.[14,15] For Mn_3O_4 and γ-MnOOH the EXAFS simulations were performed with the occupation numbers fixed at the values determined by X-ray crystallography.[8a,10a]

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Table I. Local Environment of Mn in γ- and β-MnOOH and in Mn-Reconstituted Ferritin

	γ-MnOOH crystallogr[a]		γ-MnOOH EXAFS		β-MnOOH EXAFS			Mn-ferritin EXAFS		
atom type	N^b	R/Å[c]	R/Å	$2\sigma^2$/Å^2[d]	N^e	R/Å[c]	$2\sigma^2$/Å^2[d]	N^e	R/Å[c]	$2\sigma^2$/Å^2[d]
O	2	1.85	1.87	0.003	4	1.90	0.005	4	1.91	0.010
O	2	1.96	1.95	0.004						
O	1	2.20	2.15	0.008	1	2.13	0.001	1	2.14	0.003
O	1	2.34	2.31	0.011	1	2.33	0.007	1	2.31	0.002
Mn	1	2.75	2.78	0.016	1	2.74	0.009	2	2.88	0.016
Mn	1	2.97	2.90	0.019	1	2.89	0.004			
O	4	3.53	3.62	0.053	8	3.57	0.006	9	3.64	0.031
Mn	4	3.63	3.61	0.018	4	3.43	0.012	2	3.10	0.020
Mn	4	3.81	3.79	0.025	4	3.76	0.036	4	3.94	0.022
			[f]		[f]			[f]		

[a] Reference 11*a*. [b] Occupation number fixed at value corresponding to crystallographic value. [c] Distance. [d] Debye–Waller parameter. [e] Occupation numbers allowed to vary in least-squares refinements. [f] Further backscattering contributions are clearly visible but could not be interpreted reliably.

reconstituted ferritin, the corresponding shift was +10.3 ± 0.1 eV. These values are consistent with the presence of Mn^{II} in Mn_3O_4 and Mn^{III} in β- and γ-MnOOH *and* Mn-reconstituted ferritin.

Each of Mn_3O_4, β- and γ-MnOOH, and Mn-reconstituted ferritin (see Figure 1) displays a rich Mn K-edge EXAFS, and the Fourier transforms clearly indicate backscattering contributions from shells up to *ca.* 6 Å from the metal. For Mn_3O_4 the Mn–O and Mn- - -Mn distances up to 3.4 Å are reproduced in the EXAFS simulations to within 1% of their crystallographic values;[9a] beyond 3.4 Å, the agreement of the EXAFS and crystallographic distances deteriorates to ≤3%, especially as the EXAFS is unable to resolve the individual backscattering contributions from the oxygen shells in the ranges 3.475–3.800, 4.351–4.510, and 4.680–4.852 Å. For γ-MnOOH, simulations of the EXAFS data produce Mn–O, Mn- - -Mn, and Mn- - -O distances for the 17 shells within a radius of 6.14 Å from a Mn atom which are in agreement with the crystallographic values[11a] to ≤2.5%; the most notable discrepancy is for the Mn–O distance of 2.20 Å, which is placed at 2.15 Å in the EXAFS simulation. The generally good agreement between the distances obtained from the crystallographic and EXAFS studies of Mn_3O_4 and γ-MnOOH gives confidence in the analysis of the Mn K-edge data recorded for β-MnOOH and Mn-reconstituted ferritin.

β-MnOOH was first identified by Feitknecht *et al.*,[16] who demonstrated that a material previously termed hydrohausmannite is composed of the two phases β-MnOOH and Mn_3O_4. Characterization of β-MnOOH by electron and X-ray diffraction demonstrated[10] that it possesses a distorted $Mn(OH)_2$ (pyrochroite) structure. Nevertheless, the material has remained poorly characterized. The Mn K-edge profiles of γ-MnOOH, β-MnOOH, and Mn-reconstituted ferritin are strikingly similar, indicating a very similar local environment for Mn in each phase. Interpretation of the EXAFS for β-MnOOH and Mn-reconstituted ferritin (Table I) reveals that, in both cases, the metal's local environment is very similar to that of γ-MnOOH but involves some subtle differences: (i) the inner coordination sphere of six oxygens shows the Jahn–Teller distortion associated with Mn(III), but the four inner oxygens (involved in edge-sharing between octahedra in γ-MnOOH) are *not* resolved into two distinct groups in β-MnOOH and Mn-reconstituted ferritin; (ii) the short Mn- - -Mn distances in γ-MnOOH of 2.78 and 2.90 Å, between Mn atoms in the chains of edge-sharing octahedra, become 2.74 and 2.89 Å in β-MnOOH and coalesce at 2.88 Å in Mn-reconstituted ferritin; (iii) the Mn- - -Mn distances of 3.61 and 3.79 Å in γ-MnOOH, between Mn atoms in vertex-sharing octahedra, become 3.43 and 3.76 Å in β-MnOOH and 3.10 and 3.94 Å in Mn-reconstituted ferritin.

In γ-MnOOH the MnO_6 octahedra form edge-linked chains in the *c*-axis direction which are vertex-linked in the *a* and *b* directions to adjoining chains. The Mn- - -Mn distances corresponding to the vertex-sharing of octahedra for β-MnOOH and Mn-reconstituted ferritin suggest that these structures could be derived from that of γ-MnOOH by alternate clockwise and counterclockwise rotation of the chains of edge-sharing octahedra about axes parallel to the *c*-axis—the Mn–O distances being kept essentially constant—*and* with a concomitant lengthening and shortening, respectively, of the Mn- - -Mn distances in the *a* and *b* directions. This pattern of displacement would relate γ-MnOOH to β-MnOOH and Mn-reconstituted ferritin and, ultimately, lead to the layered structure of edge-sharing octahedra in the *bc*-plane as occurs in pyrochroite.[17]

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